

RECEIVED

MAY 09 2003

TECH CENTER 1600/2900

**In the Specification:**

Please change the title from:

*marked:*

**METHOD FOR THE DETECTION AND MEASUREMENT OF HAPTEN-  
CONJUGATED (~~BIOLOGICAL~~) BINDING (~~ENTITIES~~)MATERIALS BY  
WESTERN AND DOT-BLOT USING (~~ANTI~~)-HAPTEN-RECOGNIZING  
ENTITIES (~~ANTIBODIES~~).**

to:

*clean:*

**METHOD FOR THE DETECTION AND MEASUREMENT OF HAPTEN-  
CONJUGATED BINDING MATERIALS BY WESTERN AND DOT-BLOT  
USING HAPTEN-RECOGNIZING ENTITIES.**

Please add the following paragraph to the detailed description of the invention section:

A 10. The use of the specific biotin-recognizing ability of avidin and streptavidin can be employed in the detection scheme, in place of antibodies. If the ligand carries biotin as its' hapten, it is possible to recognize and determine the amount of blot-associated hapten-ligand, using enzyme-conjugated avidin or streptavidin (as opposed to an anti-biotin antibody), followed by colorimetric, luminescent, or other detection of said enzyme, via application of the appropriate enzyme substrate. The use of biotin-conjugated anti-hapten antibodies, followed by the use of enzyme-conjugated avidin or streptavidin, followed by colorimetric, luminescent, or other detection of said enzyme

(via application of the appropriate enzyme substrate), can also be used to detect the blot-associated hapten-ligand.

Paragraph 10 in the detailed description section is now paragraph 11:

A<sup>2</sup>  
~~10~~11. Conclusion: the invention is a procedure for measuring the binding of an entity (ligand) to a surface by using a hapten-conjugated version of the ligand (hapten-ligand), where the hapten is recognizable by an antibody. An excess of the hapten-ligand is presented to the binding surface and excess (unbound) hapten-ligand is washed off. Bound hapten-ligand is then solubilized (removed) and applied to a membrane support or separated by electrophoresis and applied to a membrane support. The membrane-bound hapten-ligand is detected by application of an enzyme-conjugated antibody to the hapten; or by application of an antibody to the hapten followed by application of an enzyme-conjugated antibody to the anti-hapten antibody. The resultant membrane-associated enzyme is detected and quantitated by the application of a color or light-producing substrate which reacts with the enzyme. This assay method has the advantages of providing verification of the molecular weight of the binding substance (ligand) via the electrophoresis step. It eliminates the need for radioactive materials. The procedure provides for high sensitivity detection as the dual antibody incubation steps amplify the signal significantly. The procedure allows for easy standardization as different user-definable levels of a standard solution of the Hapten-ligand can be simultaneously applied to the electrophoresis gel or to the dot-blot or slot-blot membrane